

# THE EFFECT OF INTRAVENOUSLY-ADMINISTERED ACTH ON PLASMA 17, 21-DIHYDROXY-20-KETOSTEROIDS IN NORMAL INDIVIDUALS AND IN PATIENTS WITH DISORDERS OF THE ADRENAL CORTEX<sup>1</sup>

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The response of the adrenal cortex to exogenous ACTH has been recognized as one of the more specific and quantitative tests of adrenal function (1). Changes in the 24-hour excretion values of urinary 17-ketosteroids and 17-hydroxycorticosteroids during ACTH administration have proved to be valuable indices of adrenocortical capacity (2, 3). With the development of technics for the chemical estimation of free 17, 21-dihydroxy-20-ketosteroids in plasma (4, 5), more direct measurement of adrenal activity has become possible. Recent investigations have demonstrated the usefulness of the plasma 17-hydroxycorticosteroid response to ACTH in assessing adrenal function in normal individuals and in certain adrenocortical disorders (6-11).

Studies have been performed in this laboratory on plasma 17-hydroxycorticosteroid levels in patients with endocrine and non-endocrine diseases (12). During the course of these investigations, it became apparent that there are disease states, particularly primary adrenal insufficiency and hypopituitarism, in which plasma 17-hydroxycorticosteroid levels determined before and after ACTH administration may give a more accurate picture of adrenal capacity than single plasma 17-hydroxycorticosteroid levels. These findings prompted an evaluation of plasma 17-hydroxycorticosteroid response to ACTH in patients with a variety of disorders of the adrenal cortex as compared with normal subjects.

## METHODS AND MATERIALS

1. Plasma 17, 21-dihydroxy-20-ketosteroids were determined by the modified Silber-Porter method (5) as

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previously described (12). Normal values in this laboratory range from 4 to 32 micrograms per 100 ml. plasma (12).

2. The intravenous route of ACTH administration was selected as the most direct method of adrenal stimulation, since it avoids the variability of absorption and local tissue inactivation which have been reported to occur when ACTH is injected intramuscularly (2).

Because of the contradictory reports in the literature concerning optimal dosage and duration of administration of ACTH, it appeared impractical to aim for maximal adrenocortical stimulation or for the attainment of a steady state of steroid output (2, 11, 13). It seemed more informative to adhere to a standardized method of adrenocortical stimulation, and to compare the response of patients with disordered adrenal function with that of normal individuals under these standard conditions. The technic of the ACTH test used was as follows:

Twenty-five mgm. (25 i.u.) of ACTH (Armour ACTHAR-C, Lot R-K23601) <sup>4</sup> freshly dissolved in 500 ml. of 5 per cent dextrose and water was administered at a constant rate over a period of four hours. Twenty-five ml. blood specimens to which heparin <sup>5</sup> was added were drawn at the beginning and end of the ACTH infusion. Specimens were centrifuged, and 10 ml. plasma immediately separated and refrigerated. 17, 21-dihydroxy-20-ketosteroid determinations were performed within 72 hours. (In a few instances indicated in the Tables where the intravenous route could not be used ACTH was given by intramuscular injection.)

## CLINICAL MATERIAL

1. *Normal individuals.*

2. *Addison's disease.*

3. *Hypopituitarism.* In six of the ten patients in this group there was definite roentgenographic or operative evidence of pituitary chromophobe adenoma or of craniopharyngioma, in addition to clinical findings characteristic of hypopituitarism. One patient had a suprasellar tumor, probably a glioma of the optic chiasm. In one case, the major portion of the pituitary had been removed or destroyed during craniotomy twelve years prior to the study. One case represented an example of post-partum necrosis of the pituitary. In the tenth patient,

<sup>4</sup> ACTH used in these studies was generously supplied by Armour & Co.

<sup>5</sup> Heparin was supplied by Dr. Kenneth Thompson of Organon, Inc.

who had no demonstrable intracranial lesion, the diagnosis was based on clinical hypogonadism, hypoadrenalism, and hypothyroidism. The fact that the hypothyroidism was secondary was established by low serum precipitable iodine levels which rose to normal following the administration of thyroid-stimulating hormone.

4. *Cushing's syndrome.* In the three patients with hyperadrenalism due to bilateral adrenocortical hyperplasia, diagnosis was based on clinical and laboratory findings, and confirmed by operation and histologic study of the adrenals. In the single patient with adrenal carcinoma,<sup>6</sup> the clinical features suggesting malignancy were 1) a "mixed" clinical picture (*i.e.*, evidence of hypersecretion of both 11, 17-oxysteroids and of androgens), 2) very high urinary 17-ketosteroid and 17-OH-corticosteroid values, 3) an 8 cm. unilateral suprarenal mass,

<sup>6</sup> Studies in this patient were carried out through the courtesy of Dr. Herbert Koteen of the New York Hospital.

and 4) numerous metastatic nodules in the liver (by palpation) and lungs (by X-ray).

5. *Congenital adrenal hyperplasia.* Of the six patients studied, four were female pseudohermaphrodites, and two were males with macrogenitosomia praecox. All patients in this group excreted increased amounts of urinary 17-ketosteroids, which fell to normal levels following cortisone administration. None of the patients showed evidence of adrenal insufficiency, and none had the "salt-losing" form of the disorder.

## RESULTS

The results are summarized graphically in Figure 1.

### A. Normal subjects

Four-hour intravenous ACTH tests were performed in eleven normal individuals ranging in

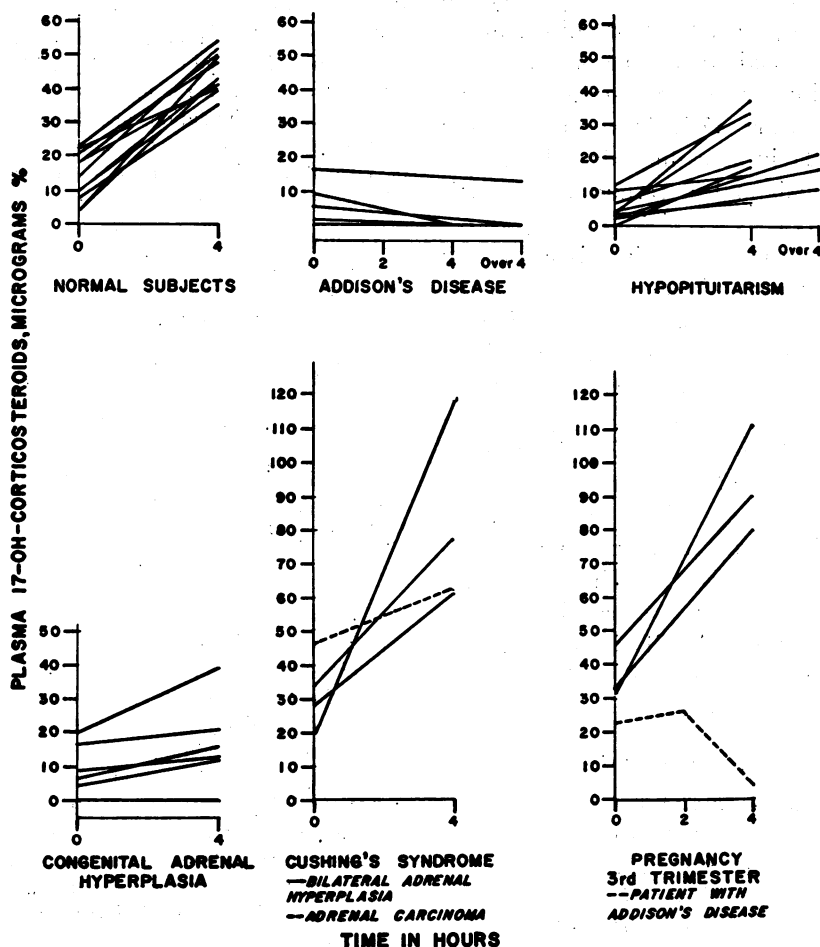


FIG. 1. EFFECT OF ACTH ADMINISTRATION ON PLASMA 17-OH-CORTICOSTEROID LEVELS IN NORMAL INDIVIDUALS AND IN PATIENTS WITH ABNORMAL ADRENOCORTICAL FUNCTION

TABLE I

*Effect of 25 mgm. ACTH administered intravenously over four hours on plasma 17-OH-corticosteroid levels of normal subjects*

Patient	Sex	Plasma 17-OH-corticosteroids, micrograms %	
		Before ACTH	After ACTH
1	♂	22	40
2	♂	4	49
3	♂	21	48
4	♀	10	39
5	♀	18	41
6	♀	8	35
7	♀	9	42
8	♀	23	54
9	♀	14	52
10	♀	18	48
11	♀	4	42

age from 14 to 59 years. The range of control values of 17, 21-dihydroxy-20-ketosteroids was 4 to 23 micrograms per 100 ml. of plasma. At the end of the ACTH infusion, values ranged from 35 to 54 micrograms per cent. There was no apparent correlation between control values and levels attained after ACTH. For example, as can be seen in Table I, patients No. 2 and No. 3 attained nearly identical plasma 17-hydroxycorticosteroid levels, despite the widely different control values (4 and 21 micrograms per cent, respectively.)

#### B. Primary adrenal insufficiency

Seven ACTH tests were performed in as many patients with documented Addison's disease. In none of these patients was a rise in plasma 17-hydroxycorticosteroid levels seen after ACTH

TABLE II

*Effect of ACTH on plasma 17-OH-corticosteroid levels in patients with Addison's disease*

Patient	Sex	Total dose mgm.	Duration of administration	Route of administration	17-OH-corticosteroids, micrograms %	
					Before ACTH	After ACTH
R. L.	♀	25	4 hrs.	Intravenous	1	0
J. B.	♂	25	4 hrs.	Intravenous	4	5
L. B.	♂	25	2 hrs.	Intravenous	0	0
F. K.	♀	25	4 hrs.	Intravenous	9	0
R. F.	♂	50	4 hrs.†	Intravenous	16	13
R. G.	♀	300	3 days	Intramuscular	1	0
C. H.	♂	300	3 days	Intramuscular	5	0
M. K.*	♀	25	4 hrs.	Intravenous	23	4

\* Pregnant Addisonian in the third trimester.

† Two four-hr. infusions administered on successive days.

administration (see Figure 1 and Table II). In four cases (patients J. B., F. K., C. H., and R. F.), the control values of plasma 17-hydroxycorticosteroids were within the normal range (12). However, the response of all four patients to ACTH administration was distinctly abnormal in that post-ACTH levels of plasma 17-hydroxycorticosteroids failed to show an increase over control values. The following is a brief summary of the findings in one of these four patients.

A 13-year old white boy (R. F.) had had numerous bouts of nausea and vomiting following minor respiratory infections since the age of 6. He had sustained two severe episodes of hypotension, one of which was followed by a prolonged period of nitrogen retention. When first seen in Babies Hospital, he was diffusely tanned, with one deeply pigmented scar. Blood pressure was 90/60, urinary corticoids<sup>7</sup> were 0.4 to 1.9 mgm. per 24 hours, and serum sodium was 128 mEq. per liter. Four-hour intravenous ACTH tests were performed on two successive days. The control plasma 17-hydroxycorticosteroid level was 16 micrograms per cent (see Table II). After the second ACTH infusion, the level was 13 micrograms per cent. On a regimen of added salt, DOC trimethylacetate, and cortisone, the patient has gained weight and felt well. Repeated serum sodium determinations have been normal.

The eighth patient, M. K., a woman with Addison's disease who was in the third trimester of pregnancy when studies were performed, had a control plasma 17-hydroxycorticosteroid value of 23 micrograms per cent. After two hours of intravenous ACTH infusion, her plasma level was 26 micrograms per cent (not considered a significant rise), and at the fourth hour, the level was 4 micrograms per cent.<sup>8</sup>

#### C. Hypopituitarism

The effect of ACTH administration was studied in ten patients with hypopituitarism (see Table III). Control plasma 17-hydroxycorticosteroid levels were low, ranging from 0 to 12 micrograms per cent. After ACTH the levels attained by most of these patients were considerably lower than those found in ACTH-stimulated normal individuals (compare Tables I and III). How-

<sup>7</sup> Total urinary corticoids determined by the Porter-Silber reaction after hydrolysis with beta-glucuronidase. Normal values, 3 to 9 mgm. per 24 hours.

<sup>8</sup> This low value may represent a technical error since the optical density of the blank was unusually high.

TABLE III  
Effect of ACTH on plasma 17-OH-corticosteroids in patients with hypopituitarism\*

Patient	Sex	Lesion	Dose ACTH mgm.	Plasma 17-OH-corticosteroids gamma %	
				Before ACTH	After ACTH
J. M.	♂	Idiopathic	475†	4	17
R. H.	♀	? Postpartum pituitary necrosis	25	4	13
T. R.	♂	Post-hypophysectomy	a) 50† b) 25‡	2 3	11 6
H. S.	♂	Chromophobe adenoma	25	0	17
J. C.	♂	Chromophobe adenoma	25	10	15
C. C.	♂	Chromophobe adenoma	75	2	21
J. J.	♂	Craniopharyngioma	25	6	19
W. R.	♂	Chromophobe adenoma	25	4	30
H. P.	♀	Chromophobe adenoma	25	12	33
M. B.	♂	Glioma, optic chiasm	25	3	37

\* ACTH administered intravenously over four hours unless otherwise indicated.

† ACTH given intramuscularly in 25 mgm. doses q6h.

‡ ACTH 25 mgm. given intravenously over four hours on two successive days.

§ This test performed 19 days after test (a).

|| ACTH 25 mgm. given intravenously over four hours on three successive days.

ever, the definite rise seen in all of these patients was in contrast to the complete lack of response to ACTH found in the patients with primary adrenocortical insufficiency (compare Tables II and III).

In three patients (W. R., H. P., and M. B.) plasma 17-hydroxycorticosteroid responses to ACTH administration approached those encountered in the normal subjects.

In seven instances, in addition to the determination of plasma 17-hydroxycorticosteroids, urinary 17-ketosteroid and "corticoid" excretion was measured during the 24-hour period which included the four-hour ACTH infusion. Increase in urinary steroid values was found in four cases (Table III, patients J. M., C. C., H. P., and M. B.), while in three patients (R. H., T. R., and W. R.) no increase in urinary values occurred.

#### D. Cushing's syndrome

ACTH tests were performed in three patients with hyperadrenalism due to bilateral adrenal hyperplasia. One patient (S. B.) was studied before surgery, and two (K. R., and C. P.) after the first but prior to a second adrenalectomy.<sup>9</sup> It will be seen in Table IV that the rises in plasma 17-hydroxycorticosteroid levels were in excess of the rises found in the normal subject (compare Table I and Table IV).

In two of the patients it was possible to assess the adrenocortical response to ACTH after bilateral subtotal adrenalectomy. K. R., in whom ACTH caused a rise in plasma 17-hydroxycorticosteroid level to 118 micrograms per cent before the second adrenalectomy, had a postoperative

<sup>9</sup> Adrenalectomies performed by Dr. George F. Cahill.

TABLE IV  
Effect of ACTH on plasma 17-OH-corticosteroids of patients with Cushing's syndrome  
(25 mgm. ACTH administered intravenously over four hours)

Patient	Sex	Lesion	Plasma 17-OH-corticosteroids, micrograms %			
			Before adrenalectomy		After adrenalectomy	
			Before ACTH	After ACTH	Before ACTH	After ACTH
S. B.	♀	Bilateral adrenal hyperplasia	34	77	31	61
K. R.*	♀	Bilateral adrenal hyperplasia	20	118	12	30
C. P.*	♂	Bilateral adrenal hyperplasia	28	61	0	8†
L. T.	♀	Adrenal carcinoma	46	62	—	—

\* These two patients had had unilateral adrenalectomy prior to ACTH administration. Operation here referred to is the second of 2 adrenalectomies.

† This patient had signs and symptoms of adrenal insufficiency following his second adrenalectomy.

TABLE V

*Effect of ACTH (25 mgm. intravenously over a four-hour period) on plasma 17-OH-corticosteroids in patients with congenital adrenal hyperplasia*

Patient	Sex	Plasma 17-OH-corticosteroids, micrograms %	
		Before ACTH	After ACTH
E. B.	♂	7	16
R. W.	♂	5	12
K. A.	♀	17	21
J. L.	♀	9	12
T. K.	♀	0	0
D. P.	♀	20	39

ACTH response of smaller magnitude than that encountered in normal individuals. In C. P., there was clinical evidence of hypoadrenalism after the second adrenalectomy when replacement therapy was withdrawn (*i.e.*, weakness, fever, hypotension, hemoconcentration, and a fall in serum sodium values from 141 to 131 mEq. per L.). At that time, he demonstrated a subnormal rise in plasma 17-hydroxycorticosteroid levels after ACTH (from 0 to 8 micrograms per cent).

A fourth patient with Cushing's syndrome due to adrenal carcinoma was studied (L. T., see Table IV). The control plasma 17-hydroxycorticosteroid level was 46 micrograms per cent, with a rise to 62 micrograms per cent after ACTH.

#### E. Congenital adrenal hyperplasia

Table V summarizes the results of ACTH administration in six patients with congenital adrenal hyperplasia, ranging in age from 6 to 18 years. There appeared to be three patterns of response. In four instances, minimal plasma 17-hydroxycor-

ticosteroid rises occurred after ACTH (see Table V, patients E. B., R. W., K. A., and J. L.). In one patient (T. K.), who exhibited no detectable plasma 17-hydroxycorticosteroid level before ACTH, there was no change. In only one patient (D. P.) was a normal response found.

Similar results have been obtained in other clinics following ACTH administration to patients with this disorder. The observations are summarized in Table VI.

#### F. Normal pregnancy

Three normal women were studied during the third trimester of pregnancy. All three showed rises of plasma 17-hydroxycorticosteroid levels in excess of the rise found in normal subjects. The control values ranged from 32 to 46 micrograms per cent. After intravenous ACTH values ranged from 79 to 111 micrograms per cent.

#### DISCUSSION

The response of plasma 17, 21-dihydroxy-20-ketosteroids to the intravenous administration of ACTH affords a means of characterizing adrenal function in various disorders of the adrenal cortex. In normal individuals, under the conditions of the standardized ACTH test used in this study, the plasma 17-hydroxycorticosteroid values following ACTH varied from 35 to 54 micrograms per 100 ml. plasma. (These values are above the range found in normal subjects without exogenous ACTH [12].) The plasma levels attained at the end of the ACTH test appeared to be more char-

TABLE VI

*Effect of ACTH on plasma 17-OH-corticosteroids in patients with congenital adrenal hyperplasia as reported by various authors*

Author	Number of patients	Plasma 17-OH-corticosteroids, micrograms %		Route and dose of ACTH administration
		Before ACTH	After ACTH	
Present study	1	20	39	Intravenous, 25 mgm., 4 hr.
	5	0-17	0-21	
Bayliss (14)	1	3	8	Intravenous, 20 mgm., 8 hr.
Bongiovanni (15)	2	3-8	20-28	Intravenous, 0.5 mgm./kgm., 3 to 4 hrs.
	7	1	No significant rise	
Ely et al. (10)	6	2.6	2.6	Intramuscular, 25 to 100 mgm., 2 hr.
Kelley et al. (9)	1	0	11.3	Intramuscular, 25 to 100 mgm.
	2	2.5-7.9	No rise	

acteristic than any set increment over the control plasma levels. This relative constancy of response suggests a certain uniformity in the secretory capacity of the normal human adrenal cortex, under the conditions of this testing method. Similar findings have been reported by Eik-Nes, Sandberg, Nelson, Tyler, and Samuels (11).

Diminished responses to intravenous ACTH were encountered in three groups of subjects: patients with 1) Addison's disease, 2) hypopituitarism, and 3) congenital adrenal virilism due to bilateral adrenal hyperplasia. In the patients with Addison's disease, ACTH consistently failed to cause a rise in plasma 17-hydroxycorticosteroid values. This was the rule whether the period of ACTH administration was brief or prolonged, and whether the control plasma 17-hydroxycorticosteroid level was low or within normal limits. The uniform failure of plasma 17-hydroxycorticosteroid levels to rise following ACTH is at variance with the findings of Eik-Nes and his co-workers (11) who noted appreciable though subnormal plasma 17-hydroxycorticosteroid rises within two hours after the start of ACTH infusions in two of six patients with Addison's disease. In this laboratory, the only patients who as a group have failed to show adrenocortical response to exogenous ACTH have been those with primary adrenal insufficiency.

In contrast to the Addisonian patients, those with hypopituitarism invariably showed an increase in plasma 17-hydroxycorticosteroid values following ACTH. However, in seven of the ten cases studied the magnitude of the increase was much smaller than that found in normal subjects.

In patients with hypopituitarism, it is relatively simple to evaluate thyroid function by means of the basal metabolic rate, the thyroïdal uptake of radioactive iodine, the plasma level of protein-bound iodine, and the response of the thyroid to stimulation by thyrotropin. Gonadotropic hypofunction can be documented both by clinical criteria and by the absence of urinary gonadotropins. The assessment of adrenocortical function and of the patient's need for adrenal replacement therapy are not as easily accomplished. The low urinary steroid excretion values and the urinary steroid response to ACTH which have been used as indices of adrenal activity in patients with hypopituitarism, may be influenced by disordered thyroid

and gonadal function as well as by the poorly-understood effects of a chronic debilitating disease. At the present time it is not possible to predict with certainty whether an individual patient with hypopituitarism will require cortisone for optimal maintenance. It may be possible when these patients have been followed for a longer period of time to correlate their need for adrenal replacement therapy with the diminished responsiveness to ACTH. Eventually then, the ACTH test may serve as a guide in planning such therapy.

The diminished response of plasma 17-hydroxycorticosteroid levels to ACTH stimulation found in most patients with congenital adrenal hyperplasia is in accord with the results of previous investigations (9, 10, 14, 15). It should be re-emphasized that none of the patients in this group had the so-called "salt-losing syndrome," and none had clinical evidence of adrenal insufficiency. The reduced adrenocortical response to ACTH appears to support the hypothesis that patients with this type of adrenal virilism have an inborn error of steroid metabolism characterized by a partial enzymatic block in the synthesis of 17-hydroxycorticosterone from its precursors (15-18).

Two groups of patients showed an exaggerated plasma 17-hydroxycorticosteroid rise after ACTH: 1) patients with Cushing's syndrome due to bilateral adrenal hyperplasia; and 2) normal women in the third trimester of pregnancy. In the patients with Cushing's syndrome, this excessive response occurred even after the removal of one of the hyperplastic adrenal glands. In two of these cases studied following *bilateral* subtotal adrenalectomy, subnormal increases in plasma 17-hydroxycorticosteroids were noted after ACTH. One patient with Cushing's syndrome due to adrenal carcinoma showed a plasma 17-hydroxycorticosteroid rise of much smaller magnitude than that found in the patients with hyperplasia. Other investigators have studied adrenocortical response to ACTH in Cushing's syndrome, using urinary steroid excretion changes as the index of activity. With this method, Laidlaw, Jenkins, Reddy, Harrison, and Thorn (19) found exaggerated responses in patients with bilateral adrenal hyperplasia, and no response in adrenal carcinoma. Kolb, Bruce, Liddle, Miller, and Forsham (20) reported one case of unilateral benign adenoma in

which excessive rises in urinary corticosteroid values were noted following ACTH administration. In Laidlaw's patients with benign adenoma, an excessive rise in urinary corticosteroids was found in one case, while three patients responded normally (19). On the basis of the data now available, the plasma corticosteroid response to ACTH clearly differentiates adrenocortical carcinoma from hyperplasia. Experience with adrenal adenomas has been too limited to permit a definite statement concerning the usefulness of the ACTH test in distinguishing Cushing's syndrome of this etiology from either of the other two categories. In the small number of cases studied postoperatively, the ACTH-corticosteroid response appears to be a useful index of the efficacy of treatment.

During the course of normal pregnancy urinary (21) and plasma (22) corticosteroid levels are known to rise progressively until term. However, the source of these "adrenal-like" steroids has not been clearly established. It is also known that urinary steroid values in patients with Addison's disease show progressive increases with advancing pregnancy (23, 24). Again, the source of these "corticoids" remains a matter of debate. In the one pregnant Addisonian patient studied in this clinic, the rise in urinary steroid excretion values was again observed, and paralleled a rise in plasma 17-hydroxycorticosteroid levels. It seemed important to determine whether the source of these substances could be stimulated by ACTH. Accordingly, a standard ACTH test was performed in the third trimester of the pregnancy. No further increase in plasma 17-hydroxycorticosteroids was demonstrated. This confirms the results of Hills, Venning, Dohan, Webster, and Richardson (24) who noted no increase in urinary "corticoids" after ACTH in two pregnant patients with Addison's disease. These findings prompted studies in normal women in the third trimester of pregnancy. The patients showed increases in plasma 17-hydroxycorticosteroids after ACTH which were far in excess of the normal.

From these data, it seems permissible to draw two conclusions: 1) The increase of Porter-Silber reactive substances, measured as plasma 17-hydroxycorticosteroids which occurred in advancing pregnancy in this patient with Addison's disease,

was due to an extra-adrenal source which could not be further stimulated by ACTH;<sup>10</sup> 2) The increasing concentration of these substances in normal pregnancy may be due both to an extra-adrenal source and to the adrenal cortex, but the excessive corticosteroid increase following ACTH must be due to an enhanced responsiveness of the adrenal cortex itself.

#### SUMMARY

The usefulness of the plasma 17,21-dihydroxy-20-ketosteroid response to intravenously-administered ACTH in the assessment of adrenocortical function has been confirmed. After a standard ACTH test, changes in plasma 17-hydroxycorticosteroid levels were rather constant in normal individuals. Under these conditions, no increase in plasma levels was encountered in patients with Addison's disease. Subnormal rises in plasma 17-hydroxycorticosteroid values were demonstrated in patients with hypopituitarism and congenital adrenal hyperplasia. Adrenocortical responses to ACTH in excess of the normal occurred in women in the third trimester of normal pregnancy and in patients with Cushing's syndrome due to bilateral adrenal hyperplasia. In the latter patients, bilateral subtotal adrenalectomy was followed by subnormal plasma 17-hydroxycorticosteroid response to ACTH.

#### Addenda

(a) Since these studies were completed Eik-Nes and his co-workers have reported data on the effects of intravenous ACTH administration on plasma 17-OH-corticosteroid levels in various non-endocrine disorders, in Addison's disease, and in one patient with Cushing's syndrome (25).

(b) Two additional patients with Cushing's syndrome due to bilateral adrenocortical hyperplasia have been tested with ACTH in the manner described, and showed a similar hyper-responsiveness.

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<sup>10</sup> The question of whether this source is a maximally-stimulated adrenocortical remnant or an extra-adrenal site (*e.g.*, the placenta) cannot at present be answered. Evidence presented elsewhere suggests that the first alternative is less likely (23, 24).

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